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Secretory Process in Brunner's Glands During Recovery From Stimulation With a Single Dose of Pilocarpine

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ABSTRACT The secretory pathway and kinetics of the secretory process were studied in Brunner's glands of mice after stimulation of secretion with a parasympatho-mimetic drug. Adult male mice were injected with pilocarpine, while unstimulated controls received saline. The animals were subsequently administered an intravenous injection of ^{3}H-threonine, and tissue was prepared for electron microscope autoradiography at intervals ranging from 5 minutes to 2 hours after injection of the radioactive precursor. Stimulation with pilocarpine resulted in discharge of secretory granules, which was reflected in a significantly lower percentage of the cell volume occupied by granules. In both control and stimulated mice, at 5 minutes after injection of ^{3}H-threonine, the highest percentage of silver grains was found over the rough endoplasmic reticulum. The proportion of silver grains over the rough endoplasmic reticulum declined at later intervals, and a peak of labeling was observed over the Golgi apparatus at 1 hour. Labeling of the secretory granules increased in the 1- and 2-hour samples from both control and stimulated mice, although the relative concentration of radioactivity in both Golgi-associated and apical secretory granules was greater in stimulated than control glands at 1 hour. The results suggest that the secretory protein produced by Brunner's glands was synthesized by the rough endoplasmic reticulum and transported to the Golgi apparatus where secretory granules were formed in both stimulated and control glands. Depletion of secretory granules by prior stimulation resulted in no change in the kinetics of arrival of radioactivity in the cell organelles involved in the secretory process. However, the drainage of the radioactive label from the rough endoplasmic reticulum was significantly slower in the stimulated glands than in the controls. The existence of two subcompartments within the rough endoplasmic reticulum is among the possible explanations for the latter observation.

Normal protein-secreting cells have been studied frequently. The ultrastructure of the secretory cells of a variety of exocrine glands has been described, and cell fractionation and autoradiography have been used to analyze the intracellular pathway and kinetics of the secretory process (e.g., Siekevitz and Palade, 1960; Neutra and Leblond, 1966; Jamieson and Palade, 1967; Palade, 1975). However, much less attention has been paid to possible alterations in the secretory pathway under different conditions, such as stimulation of secretory activity.

Effects of stimulation on the morphology of secretory cells have been reported, but the results have varied. Generally it is agreed that stimulation causes degranulation by fusion of the secretory granule membrane with the apical plasmalemma (Okano and Nakamura, 1965; Bogart, 1975; Bieger et al., 1976; Helander, 1978; Fleming et al., 1980). Some investigators have found no significant ultrastructural changes in stimulated secretory cells besides depletion of granules (Sjostrand and Hanzon, 1954a,b; Bannasch and Thoenes, 1965; Ichikawa, 1965; Nevalainen, 1970; Fleming et al., 1980). Others, however, have described a variety of ultrastructural changes in the rough endoplasmic reticulum,

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the Golgi apparatus, and lysosomes (Kern and Kern, 1969; Jamieson and Palade, 1971; Lillie and Han, 1973; Volkl et al., 1976; Slot and Geuze, 1979), as considered in more detail in the Discussion.

The kinetics of protein synthesis and intracellular transport after stimulation have been studied in only a few instances, and the results have been contradictory. Two authors found no change in kinetics (Jamieson and Palade, 1971; Singh et al., 1973), but two others reported more rapid intracellular transport with stimulation (Bieger et al., 1976; Slot et al., 1979).

In the present study, Brunner's glands were examined during their recovery from stimulation of secretion by a single dose of pilocarpine (a parasympatho-mimetic drug). Previously stimulated glands were compared to nonstimulated glands to determine if depletion of secretory granules by stimulation resulted in changes in the pathway and/or kinetics of the secretory process.

The Brunner's glands are found in the submucosa of the duodenum and secrete into the crypts of Lieberkuhn a clear viscous fluid that contains carbohydrate and protein moieties. They are stimulated to secrete by nervous (parasympathetic), humoral (secretin), and mechanical (food, etc.) stimuli (Kuntz, 1953; Friend, 1965; Lang and Tansey, 1980). Their principal functions are believed to be lubrication and protection of the mucosa from the acid juices of the stomach (Leeson and Leeson, 1967). Brunner's glands are found only in mammals, and they vary with the species. Most consist of mucous-type cells (Grossman, 1958; Moe, 1960; Leeson and Leeson, 1966, 1967). The Brunner's glands of the mouse are considered to be intermediate between mucous and serous types (Friend, 1965). Ultrastructural studies have shown that the organelles associated with protein secretion such as rough endoplasmic reticulum, the Golgi apparatus, and secretory granules, are well developed in these glands (Friend, 1965; Rohr et al., 1967).

MATERIALS AND METHODS

Pilot Studies

A preliminary study was performed to test the effectiveness of ^{3}H-threonine (ICN Chemical, Radioisotope Div., specific activity 5 Ci/mmole) and ^{3}H-leucine (Amersham, specific activity 57.1 Ci/mmole) as precursors to secretory protein in murine Brunner's glands. Threonine was tested because it constitutes 20% of the amino acids in mucus (Havez and Biserte, 1969), while leucine is a common amino acid that is frequently used in studies of protein secretion. In each case, the amino acid in 0.2 ml aqueous solution was injected into the jugular vein of young adult male mice (approximately 35 gm body weight) in a dose of 1.25 mCi or 2.5 mCi. Two animals were sacrificed 1 hour later, and the proximal duodenum was excised, diced, and fixed in 1.33% OsO_4 in collidine buffer to avoid the nonspecific binding of free amino acids caused by glutaraldehyde (Peters and Ashley, 1967). The tissue was then rinsed with cacodylate buffer, dehydrated, and embedded in araldite. Sections 1 μm thick were cut with glass knives and mounted on glass slides.

Light microscope autoradiographs were prepared by coating the slides with Kodak NTB-2 emulsion diluted to half strength with distilled water. The slides were dried and stored in light-tight boxes for 1–4 weeks. The preparations were developed in Dektol, rinsed with distilled water, and fixed. After being rinsed and dried, the slides were stained with 0.25% Azure II in 0.5% sodium borate. Upon examining light autoradiographs exposed for the same length of time, it was obvious that the Brunner's glands were more heavily labeled with ^{3}H-threonine than with ^{3}H-leucine and thus ^{3}H-threonine was selected for the main experiment.

In a second pilot study, the appropriate dose of pilocarpine necessary to elicit secretory granule discharge was examined. A subcutaneous injection of pilocarpine nitrate in 0.1 ml normal saline was administered to two fasted mice at each of the test doses of 2.2, 4.4, 8.8, and 13.2 mg/100 gm body weight. Three controls were administered saline only. After 3 hours the animals were sacrificed. A 3-hour interval was used to allow the lumen to return to normal size after being enlarged by the discharging granules (Okano and Nakamura, 1965; Amsterdam et al., 1969; Jamieson and Palade, 1971; Geuze and Poort, 1973; Geuze and Kramer, 1974). The proximal duodenum was then excised, diced, fixed with a glutaraldehyde-paraformaldehyde fixative (Karnovsky, 1965), and embedded in araldite as described previously. Silver- to gold-colored thin sections were cut with a diamond knife and mounted on copper grids. Electron micrographs were taken on a Philips EM-300.

Morphometric analysis was performed on the Brunner's glands using a point hit method (Chalkley, 1943) to determine the

percentage of the cytoplasmic volume occupied by secretory granules. In mice administered the highest dose, 13.2 mg/100 gm, the volume percentage of cytoplasm occupied by secretory granules was 5.0 ± 0.1 (mean ± SEM), which was significantly less than the 14.0 ± 0.8 observed for the controls ($P < 0.005$). Since the values for animals treated with lower doses did not differ significantly from the controls, 13.2 mg/100 gm was used in the subsequent experiments.

Stimulation and Administration of ^{3}H-Threonine

Mature male Swiss white mice were fasted overnight. Secretion was stimulated by a subcutaneous injection on the back of pilocarpine nitrate (13.2 mg/100 gm b.w. in 0.1 ml normal saline). Nonstimulated controls received a subcutaneous injection of saline. Three hours later the mice were anesthetized with Penthrane and were administered 2.5 mCi ^{3}H-threonine in 0.2 ml aqueous solution by injection into the jugular vein. Two stimulated and two nonstimulated animals were sacrificed at each of the following intervals after the injection: 5, 15, and 30 minutes; 1 and 2 hours. Tissue from the proximal duodenum was fixed and prepared for light microscope autoradiography as described above.

For electron microscope autoradiography, thin sections were mounted on grids which were then dipped in a 0.05% solution of SDS (lauryl sulfate). The sections were coated with a monolayer of Ilford L-4 emulsion by the loop method (Stevens, 1966). An undeveloped coated grid was periodically examined during each coating operation to ensure that an even monolayer had been obtained. The grids were then stored in light-tight boxes for 3–8 months. The preparations were developed for 5 minutes in Kodak Microdol-X, placed for 30 seconds in 1% acetic acid, and fixed with Kodak rapid fix. They were stained with saturated uranyl acetate in 50% ethanol, followed by lead citrate (Reynolds, 1963). Electron micrographs were taken with a Philips EM-300 electron microscope.

The distribution of silver grains over the different cellular organelles was determined for each time interval for both stimulated and control animals. Silver grains were counted using a 50% probability circle of radius 6 mm centered over the silver grains (Salpeter and McHenry, 1973) on micrographs with a magnification of 22,200. If only one organelle was in the circle (an "exclusive" grain), that organelle received a count of one. If more than one cellular component lay within the circle (a "shared" grain), each received an equal fraction of the silver grain. Only micrographs of entire cells including a nucleus and an apex bordering a lumen were used. This is the preferred method of selecting micrographs of polarized cells such as those found in the Brunner's glands for analysis because it ensures equal representation of basal and apical parts of the cells, in which there are different distributions of cellular organelles (Geuze and Kramer, 1974; Helander, 1978; Ermak and Rothman, 1981). Approximately 500 silver grains were counted for each animal. The percentage of silver grains for the individual cell components was calculated by dividing the number of silver grains for that component by the total number of silver grains and multiplying by 100.

A check for background in the electron microscope autoradiographs was performed by taking pictures of sections that contained only plastic or debris and that had been exposed for the longest time. The resulting background was less than 0.11 silver grains/100 μm^2, which was considered negligible.

The percentage of the cell volume occupied by the various organelles in the autoradiographs analyzed at each interval was determined by the point hit method (Chalkley, 1942). The percentage of silver grains over an organelle was then divided by the percentage cell volume occupied by that organelle to obtain the relative grain concentration, which reflects the relative concentration of the radioactivity in that organelle (Feeney and Wissing, 1972).

RESULTS

Fine Structure

Nonstimulated controls

The pyramidal cells of the murine Brunner's glands had ultrastructural features typical of protein secreting cells (Figs. 1–3). The rough endoplasmic reticulum comprised both individual cisternae and parallel stacks (Figs. 1, 3). Although principally located at the base of the cell, some was also found apical to the nucleus. The extensive supranuclear Golgi apparatus was comprised of vesicles, stacked cisternae, and condensing vacuoles. The cisternae were usually crescent-shaped in section, with an outer, convex "cis" side and an inner, concave, "trans" side (Figs. 1, 2). Small dense secretory granules were found within the crescent defined by

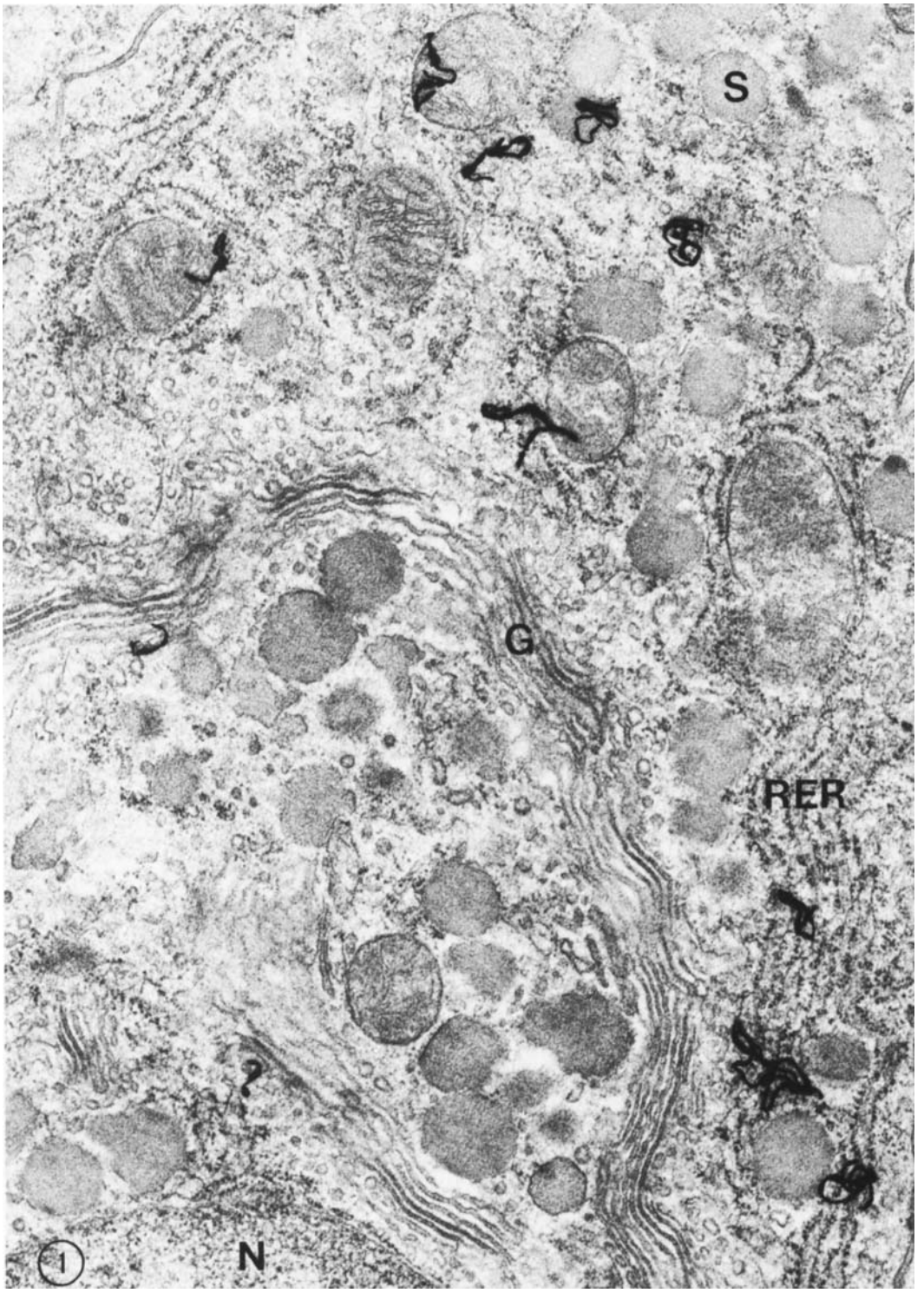

Fig. 1. Nonstimulated control 5 minutes after the administration of ^{3}H-threonine. At this interval, the highest concentration of silver grains is found over the rough endoplasmic reticulum (RER). The Golgi apparatus (G) lies above the nucleus (N) and the secretory granules (S) are at the apical end of the cell. × 34,000.

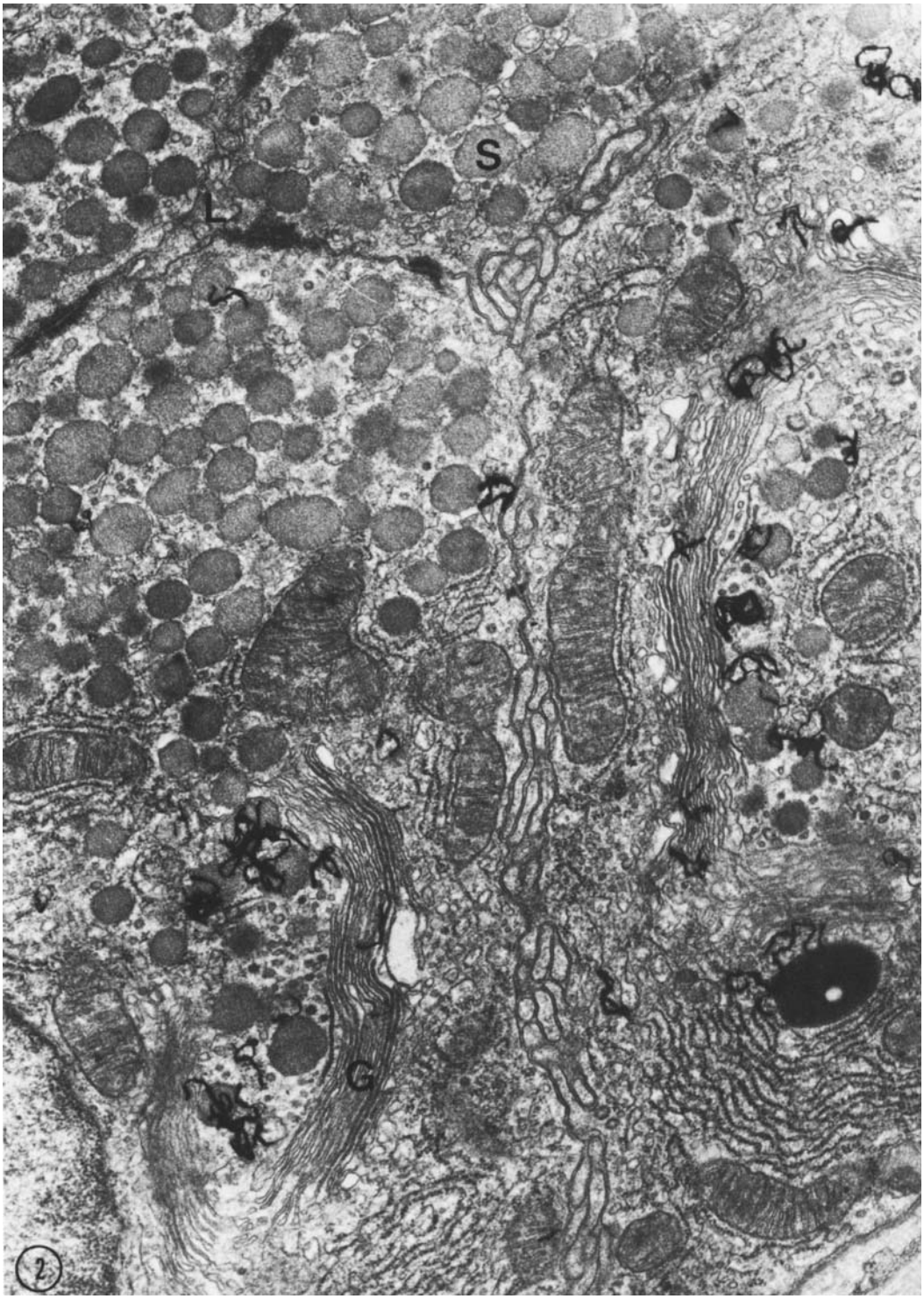

Fig. 2. Nonstimulated gland at the 1-hour interval. The Golgi apparatus (G) and Golgi-associated secretory granules are heavily labeled. There are occasional grains over the secretory granules (S) at the apical end of the cell near the lumen (L). × 29,000.

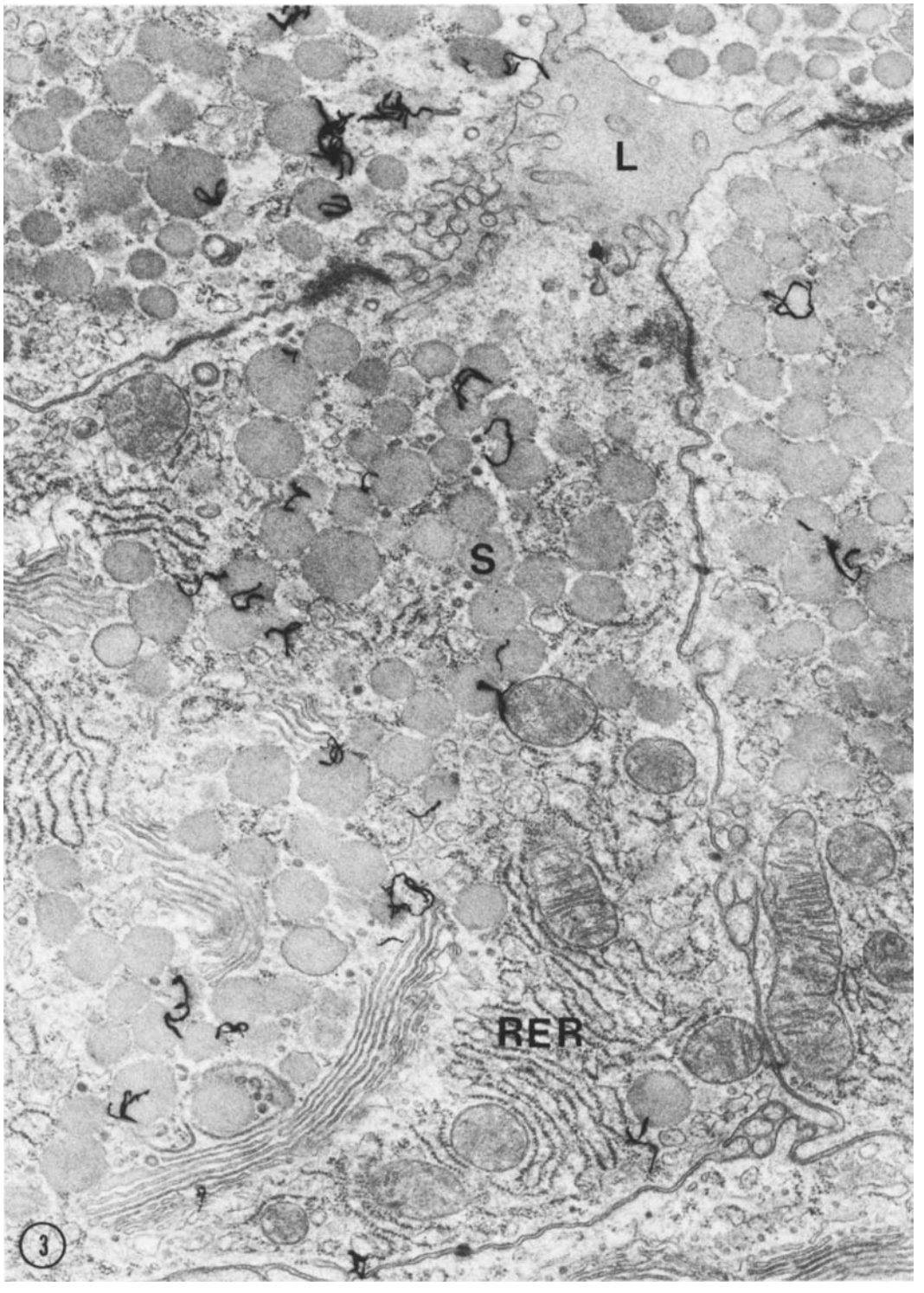

Fig. 3. Control cell 2 hours after the administration of ^{3}H-threonine. The silver grains are located over the apical secretory granules (S) near the lumen (L), as well as over the Golgi-associated secretory granules. Some grains are found over the RER and Golgi apparatus (G) although to a lesser extent than at 1 hour. × 23,500.

the Golgi apparatus and at the apical end of the cell (Figs. 2, 3). Mitochondria were numerous and frequently lay near cisternae of rough endoplasmic reticulum. Occasional lipid droplets and lysosomes were also present.

Stimulated animals

The fine structure of cells from stimulated animals (Figs. 4, 5) closely resembled that of nonstimulated controls, with one important exception. Following the administration of secretogogue, the percentage of cell volume occupied by secretory granules was reduced from 10.4 to 4.8% in the initial sample, and remained lower in stimulated than in control glands throughout the experiment (Table 1; cf. Figs. 3, 4). No morphological changes were observed in the Golgi apparatus, rough endoplasmic reticulum, or lysosomes after stimulation.

Light Microscope Autoradiography

Light autoradiographic slides were primarily used to estimate the length of time necessary for adequate electron microscope autoradiographic labeling. Some differences in the location of the label with time were noticed, suggesting a basal to apical movement of radioactive material, but the resolution was not sufficient for study of details of the secretory process.

Electron Microscope Autoradiography

Sample electron microscope autoradiographs are illustrated by Figures 1–5. The following description is based mainly on the quantitative analysis as presented in Tables 2–5 and in the graphs (Figs. 6–8).

Nonstimulated controls

The percentage of silver grains over the various organelles of nonstimulated glands (Table 2, Fig. 6) changed with time after the administration of the radioactive precursor. In the 5-minute sample (Fig. 1), about half of the silver grains were over the rough endoplasmic reticulum. Much smaller percentages of silver grains were associated with the Golgi apparatus, nucleus, mitochondria, and other cellular organelles. At 15 and 30 minutes after administration of the precursor, the percentage of silver grains over the rough endoplasmic reticulum declined, but the values were still higher than over any other cell compartment. During this time, the percentage of silver grains associated with the Golgi apparatus (Fig. 2) increased, until at 1 hour the highest percentage of silver grains was associated with the Golgi apparatus. In addition, at 1 hour there was a substantial increase in silver grains over the secretory granules, and at 2 hours the secretory granules (Fig. 3) had the highest percentage of silver grains.

The relative grain concentrations (% grains/% volume) for the nonstimulated cells (Table 3) revealed a picture similar to that of the percentage of silver grains. The rough endoplasmic reticulum had a high value at 5 minutes and started to decline thereafter, reaching a low value of under one at 2 hours. The Golgi complex started with a low value and increased with time, peaking at 1 hour. The secretory granules increased their relative grain concentration with time to a high value of more than 8, which was reached at 2 hours.

Other cellular components that appeared to undergo changes were the mitochondria and the basal and lateral plasma membranes, for which the percentage of grains and the relative grain concentrations decreased with time. The labeling of these compartments may be attributed in part to scatter from the nearby rough endoplasmic reticulum, which decreased in radioactivity with time. The apical plasma membrane showed increased labeling at 2 hours which may reflect the proximity of apical secretory granules and the lumen, which were increasingly labeled at later intervals.

Stimulated animals

At early intervals after the administration of the precursor the distribution of the percentage of silver grains in previously stimulated cells (Table 4, Fig. 6) was similar to that of the nonstimulated state. The rough endoplasmic reticulum (Fig. 4) was associated with almost 50% of the silver grains in the 5-minute sample, while the Golgi apparatus, nucleus, and other cellular compartments showed considerably less label. At 15 and 30 minutes, the silver grains over the rough endoplasmic reticulum declined as the percentage increased over the Golgi apparatus. Like the nonstimulated cells, the percentage of silver grains peaked at 1 hour over the Golgi apparatus, but the peak was not as high (Fig. 6). The secretory granules (Fig. 5) showed a pronounced rise in percentage of silver grains at 1 hour. In contrast to nonstimulated cells, the percentage of silver grains over the secretory granules remained almost constant from 1 to 2 hours, but it should be noted that a further rise at 2 hours may be masked by one unusually low value

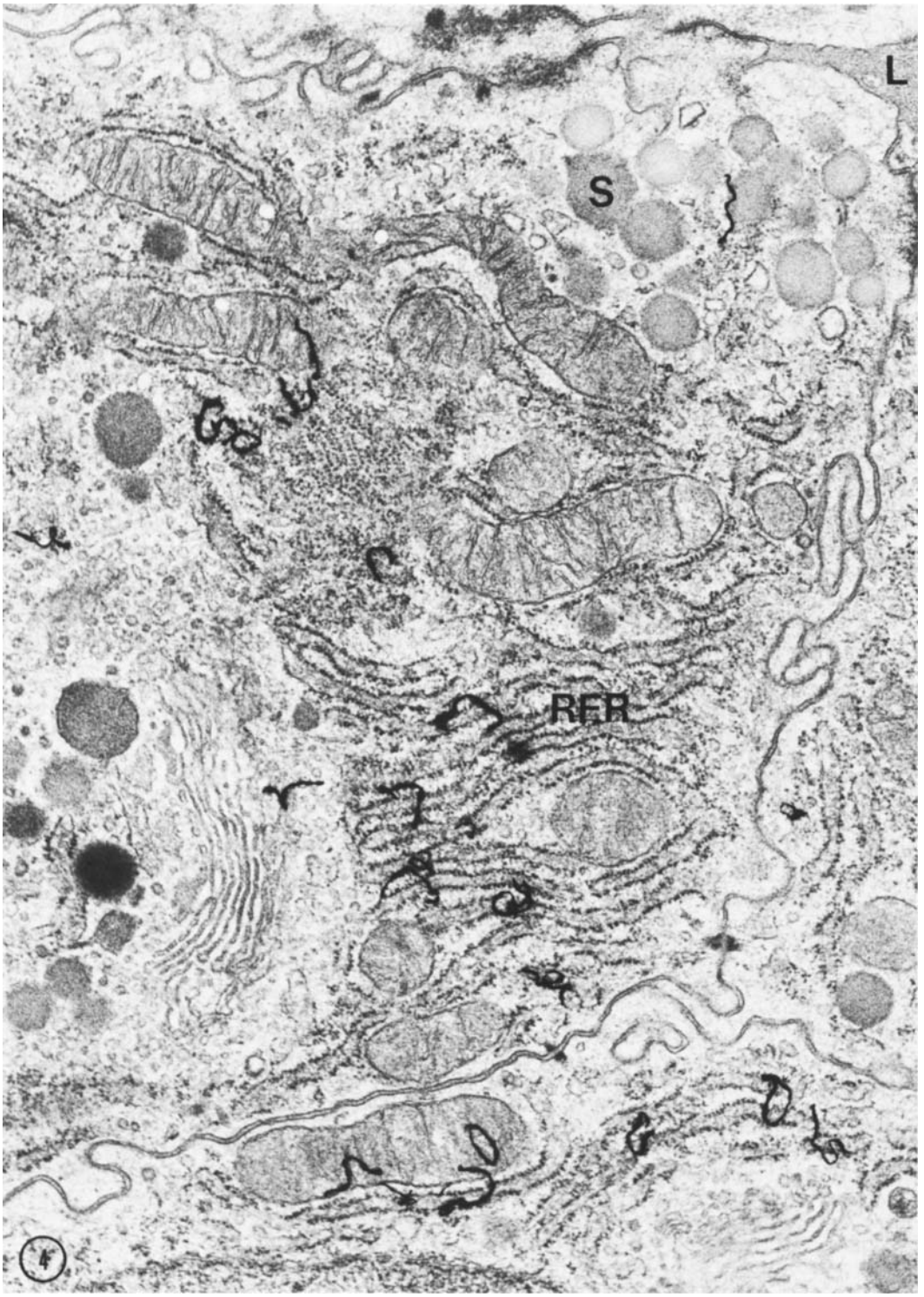

Fig. 4. Stimulated gland 5 minutes after administration of ^{3}H-threonine. The silver grains are most concentrated over the rough endoplasmic reticulum (RER). A few secretory granules (S) are located next to the Golgi apparatus (G) and the lumen (L). × 22,000.

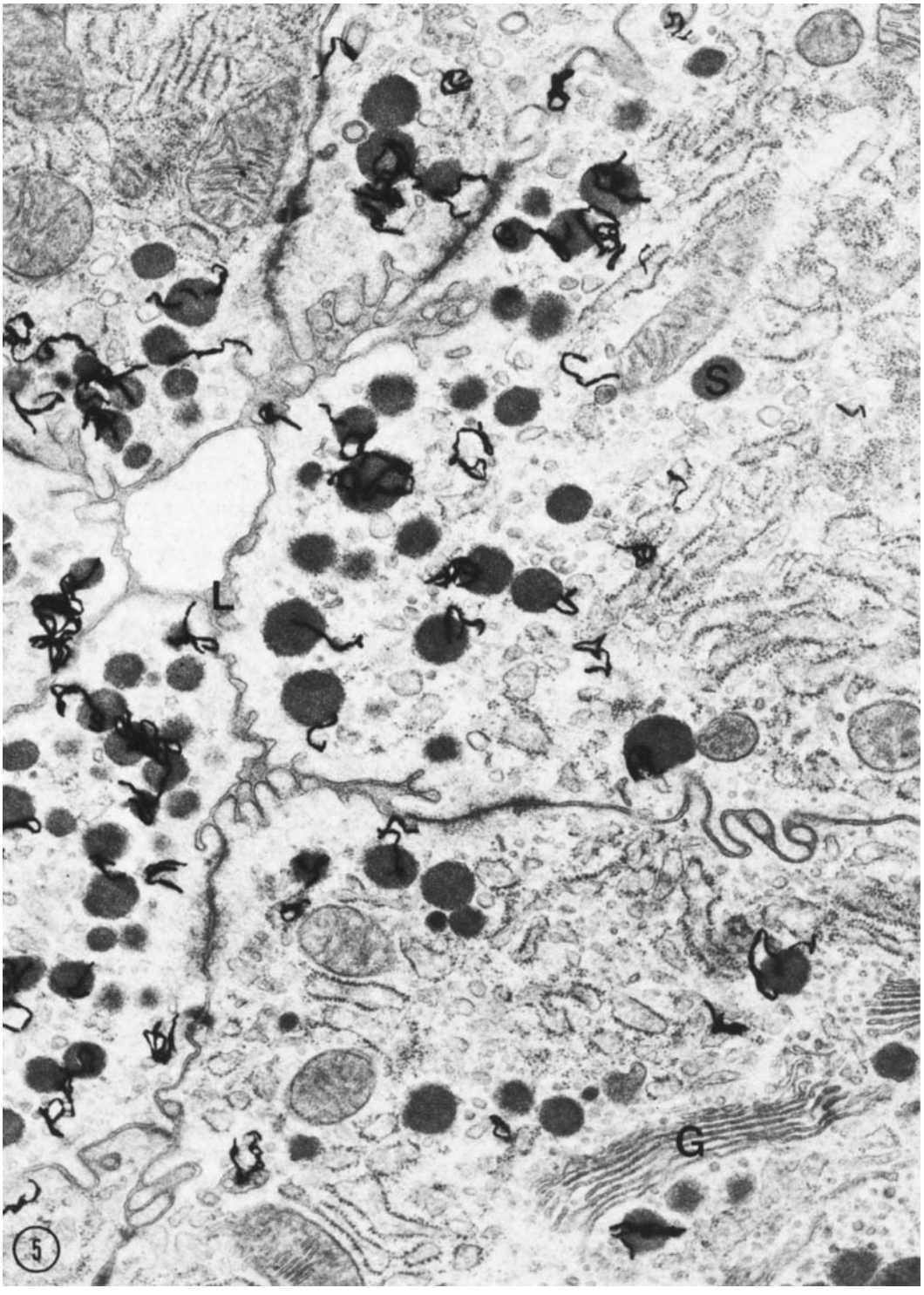

Fig. 5. Stimulated cells 2 hours after the administration of ^{3}H-threonine. Silver grains are numerous over the secretory granules (S). Note the reduced number of secretory granules located next to the lumen (L) in the stimulated state compared to the nonstimulated state (cf. Fig. 3). × 22,000.

TABLE 1. Percentage of cell volume occupied by secretory granules in autoradiographs of control (nonstimulated) and stimulated cells of Brunner's glands[1]

	5 min	15 min	30 min	1 hr	2 hr
Control	10.4 ± 2.0	11.5 ± 2.4	8.2 ± 1.2	13.1 ± 0.3	6.0 ± 2.4
Stimulated	4.8** ± 1.1	2.9* ± 0.8	5.0** ± 0.2	2.8*** ± 0.3	2.9 ± 1.3

[1]The means ± SEM are shown. The significance of the differences between means is indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001.

obtained (note in Table 4 the large standard error). At 2 hours, the percentages of silver grains associated with the Golgi apparatus and the rough endoplasmic reticulum of stimulated cells were less than at earlier intervals but did not decline to the control levels. For the rough endoplasmic reticulum the 2-hour values for control (20.1%) and stimulated cells (27.8%) were significantly different ($P > 0.05$).

The relative grain concentrations (Table 5) showed trends similar to those seen in the percentage of silver grains. Initially the rough endoplasmic reticulum had high values which declined with time, although again not to control levels. The Golgi apparatus peaked at 1 hour and was on the decline by 2 hours. The secretory granules, lumen, and apical plasmalemma increased from low to higher values by 1 hour.

Secretory granules

In an effort to follow the fate of the radioactive material within the population of secretory granules more closely, they were divided into two groups on the basis of their location. One group, designated Golgi-associated granules, consisted of secretory granules within the crescent formed by the curved stacks of Golgi cisternae as well as granules that directly bordered the Golgi complex. All other secretory granules were classified as apical secretory granules because of their location between the Golgi apparatus and the cell apex.

Analysis of electron microscope autoradiographs showed that the Golgi-associated granules were labeled before the apical granules in control cells (Fig. 7). Although the percentage of silver grains over the Golgi-associated secretory granules peaked at 1 hour after the injection of ^{3}H-threonine and that over the apical secretory granules was highest at 2 hours in both the stimulated and nonstimulated cells, the percentage of grains over both categories of granules in stimulated cells did not reach the levels observed for controls.

The Golgi-associated granules had their highest relative grain concentration (% grains/% volume) at 1 hour in both the control and stimulated cells (Fig. 8). However, in the nonstimulated controls, the apical secretory granules had a lower relative grain concentration than the Golgi-associated granules prior to the 2-hour interval, while in the stimulated cells the relative grain concentration of the apical secretory granules peaked at 1 hour and was much higher than in the nonstimulated controls.

DISCUSSION

Ultrastructural Observations

The observation that stimulation resulted in loss of secretory granules is in agreement with the findings of other authors (Sjostrand and Hanzon, 1954a,b; Ichikawa, 1965; Bannasch and Thoenes, 1965; Nevalainen, 1970; Fleming et al., 1980). In contrast, while no other ultrastructural changes were observed in the cells of Brunner's glands in the present study, some investigators have described changes in various organelles following stimulation with a secretogogue in other organs. For example, the Golgi apparatus has frequently been reported to be enlarged with stimulation (Farquhar and Welling, 1957; Bogart, 1975; Lillie and Han, 1973; Volkl et al., 1976), including an increase in the number of cisternae (Ribet et al., 1969; Jamieson and Palade, 1971) or dilation of cisternae (Hermodson, 1962; Kern and Kern, 1969; Slot and Geuze, 1979), although few of the reported changes in the Golgi apparatus were documented by morphometric analysis. The reasons for these differences from the present study are not clear, but possible explanations include different tissues, stimulus, species, time elapsed after stimulation, the in vitro nature of some studies, etc.

Changes in the rough endoplasmic reticulum have also been reported after stimula-

TABLE 2. Percentage of silver grains overlying parts of nonstimulated Brunner's gland cells at intervals after the administration of ^{3}H-threonine[1]

Organelle	5 min	15 min	30 min	1 hr	2 hr
RER	44.7 ± 3.6	40.8 ± 6.2	34.5 ± 3.7	20.4 ± 3.5	20.1 ± 0.6
Golgi	10.7 ± 2.5	21.3 ± 1.1	21.2 ± 0.1	37.7 ± 2.6	17.2 ± 2.0
Secretory granules	6.0 ± 1.1	8.4 ± 2.2	9.5 ± 0.3	23.6 ± 2.6	41.8 ± 1.0
Lumen	0.2 ± 0.2	0	0.3 ± 0.1	0.1 ± 0.03	0.7 ± 0.3
Apical P.M.	0.1 ± 0.06	0	0.2 ± 0.1	0.2 ± 0.07	1.2 ± 0.5
Basal and lateral P.M.	9.2 ± 0.9	9.1 ± 1.7	8.8 ± 1.5	4.5 ± 1.0	6.2 ± 0.7
Cytoplasmic matrix	0.5 ± 0.5	0.4 ± 0.2	0.1 ± 0.1	0	0.1 ± 0.1
Nucleus	15.1 ± 1.8	9.6 ± 1.1	14.0 ± 1.7	9.5 ± 0.2	7.3 ± 3.6
Mitochondria	12.8 ± 2.9	9.8 ± 0.2	11.0 ± 0.5	4.6 ± 0.5	5.0 ± 0.2
Other	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.6	0.3 ± 0.2	0.5 ± 0.1

[1]RER, rough endoplasmic reticulum; P.M., plasma membrane. The means ± SEM are shown.

TABLE 3. Relative grain concentrations associated with parts of nonstimulated Brunner's gland cells at intervals after the administration of ^{3}H-threonine[1]

Organelle	5 min	15 min	30 min	1 hr	2 hr
RER	3.3 ± 1.0	2.7 ± 0.8	2.3 ± 0.1	1.1 ± 0.1	0.9 ± 0.04
Golgi	0.8 ± 0.2	1.3 ± 0.5	1.3 ± 0.05	2.3 ± 0.2	0.9 ± 0.04
S.G.	0.6 ± 0	0.7 ± 0.04	1.2 ± 0.1	1.8 ± 0.2	8.2 ± 3.1
Lumen	0.9 ± 0.7	0	0.6 ± 0.02	1.1 ± 0.9	2.0 ± 0.5
Apical P.M.	1.0 ± 0.3	0	2.0 ± 1.8	0.7 ± 0.7	3.2 ± 0.7
Basal and lateral P.M.	2.4 ± 0.4	2.8 ± 0.6	2.5 ± 0.1	1.3 ± 0.5	1.7 ± 0.3
Cytoplasmic matrix	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0	0.01 ± 0.01
Nucleus	0.6 ± 0.1	0.5 ± 0.03	0.6 ± 0.1	0.4 ± 0.01	0.3 ± 0.1
Mitochondria	1.7 ± 0.3	1.4 ± 0.1	1.5 ± 0.03	0.8 ± 0.1	0.6 ± 0.05
Other	0.3 ± 0.1	0.4 ± 0.05	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0

[1]RER, rough endoplasmic reticulum; S.G., secretory granules; P.M., plasma membrane. The mean for two animals at each interval is shown ± SEM.

TABLE 4. Percentage of silver grains overlying parts of stimulated Brunner's gland cells at intervals after the administration of ^{3}H-threonine[1]

Organelle	5 min	15 min	30 min	1 hr	2 hr
RER	45.2 ± 1.8	37.5 ± 3.8	37.4 ± 7.6	29.4 ± 1.7	27.8 ± 1.8
Golgi	13.9 ± 0.2	19.8 ± 0.2	27.0 ± 7.8	30.9 ± 4.0	25.7 ± 0.7
Secretory granules	3.3 ± 0.4	5.0 ± 0.8	5.7 ± 0.3	14.9 ± 2.0	14.0 ± 8.2
Lumen	0	0.4 ± 0.1	0.3 ± 0.3	0.6 ± 0.2	0.8 ± 0.2
Apical P.M.	0.1 ± 0.02	0.7 ± 0.2	0.5 ± 0.4	1.5 ± 0.6	1.5 ± 0.3
Basal and lateral P.M.	8.4 ± 1.2	10.4 ± 1.3	7.8 ± 0.6	5.6 ± 1.6	7.6 ± 3.2
Cytoplasmic matrix	0.3 ± 0.1	2.5 ± 0.3	0.9 ± 0.9	0.4 ± 0.1	0.1 ± 0.1
Nucleus	15.2 ± 0.9	11.2 ± 1.5	10.1 ± 0.4	9.5 ± 5.0	14.2 ± 1.5
Mitochondria	12.7 ± 0.4	11.8 ± 0.5	9.9 ± 2.1	6.7 ± 1.8	7.5 ± 1.2
Other	0.8 ± 0.04	0.8 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.7 ± 0.5

[1]RER, rough endoplasmic reticulum; P.M., plasma membrane. The means ± SEM are shown.

TABLE 5. Relative grain concentrations associated with parts of stimulated Brunner's gland cells at intervals after the administration of ^{3}H-threonine[1]

Organelle	5 min	15 min	30 min	1 hr	2 hr
RER	2.7 ± 0.4	2.8 ± 0.01	2.4 ± 0.3	1.7 ± 0.2	1.4 ± 0.05
Golgi	0.8 ± 0	1.2 ± 0.04	2.0 ± 0.9	2.0 ± 0.1	1.3 ± 0.1
S.G.	0.7 ± 0.1	1.8 ± 0.2	1.1 ± 0.02	5.6 ± 1.2	4.4 ± 0.9
Lumen	0.3 ± 0.2	0.6 ± 0.04	0.2 ± 0.2	3.3 ± 1.2	3.1 ± 1.4
Apical P.M.	0.3 ± 0.1	1.2 ± 0.2	0.8 ± 0.4	7.3 ± 5.8	3.9 ± 0.5
Basal and lateral P.M.	2.0 ± 0.5	2.7 ± 0.4	1.8 ± 0.6	1.1 ± 0.2	1.6 ± 0.4
Cytoplasmic matrix	0.02 ± 0.01	0.1 ± 0.01	0.03 ± 0.03	0.4 ± 0.3	0.01 ± 0.01
Nucleus	0.7 ± 0.05	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.1
Mitochondria	1.7 ± 0.1	1.8 ± 0.04	1.6 ± 0.05	1.0 ± 0.3	1.1 ± 0.1
Other	2.2 ± 1.1	0.2 ± 0.02	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1

[1]RER, rough endoplasmic reticulum; S.G., secretory granules; P.M., plasma membrane. The means of two animals are shown ± SEM.

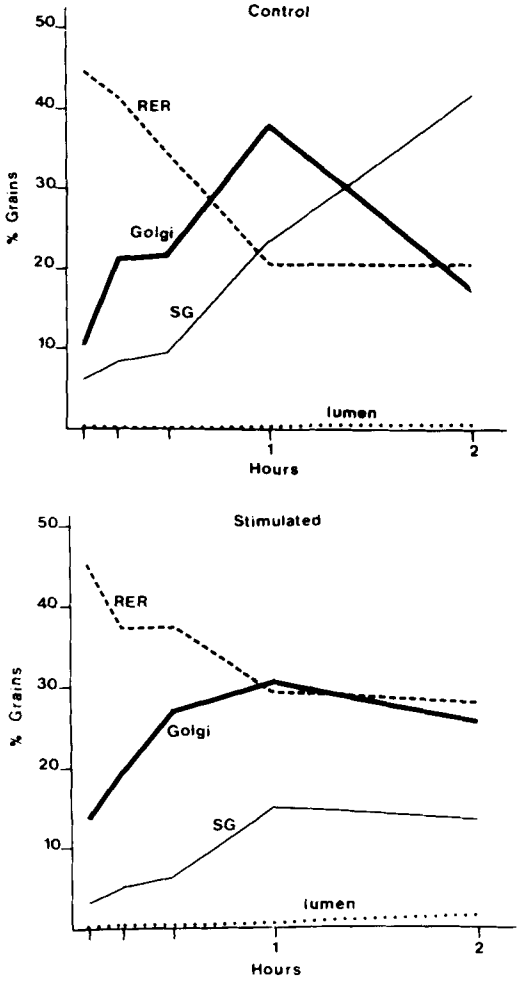

Fig. 6. The percentage of silver grains over selected compartments of control and stimulated Brunner's gland cells at intervals after injection of ^{3}H-threonine. The percentage of grains over the remaining parts of the cells is shown in Tables 2 and 3. RER, rough endoplasmic reticulum; SG, secretory granules.

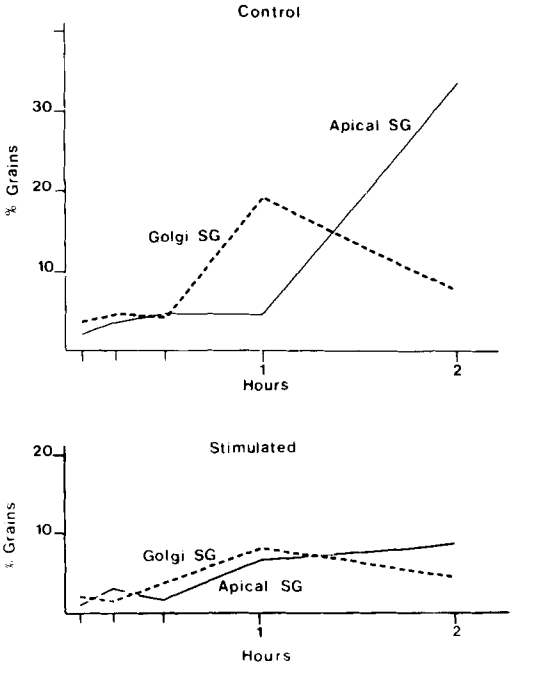

Fig. 7. The percentage of silver grains overlying different populations of secretory granules in control and stimulated animals. Golgi SG, Golgi-associated secretory granules; apical SG, apical secretory granules.

tion in other systems. Some early descriptions of the rough endoplasmic reticulum becoming vesicular (Palay, 1958; Okano and Nakamura, 1965) or undergoing breakdown (Lacy, 1955) with stimulation may be related to the degree of tissue preservation. Upon stimulation, the rough endoplasmic reticulum also has been reported to have dilated cisternae (Kern and Kern, 1969; Simson et al., 1978; Slot and Geuze, 1979), increased separation of cisternae (Oron and Bdolah, 1973; Slot and Geuze, 1979; Cope and Williams, 1980), increased numbers of intracisternal granules (Jamieson and Palade, 1971), and unusual cisternae that converge and diverge (Lillie and Han, 1973), but the relation of these findings to the dynamics of the secretory process is problematic.

Route of the Secretory Proteins

No changes in the intracellular route of secretory protein in Brunner's glands were observed after stimulation. The localization of nearly 50% of the radioactivity in the rough endoplasmic reticulum at the initial interval in both the stimulated and nonstimulated cells is consistent with findings in other protein-secreting glands (Palade, 1975) and reflects protein synthesis in this location in Brunner's glands. The subsequent sequential labeling of the Golgi apparatus and secretory granules most likely indicates that protein was transported to these organelles from the rough endoplasmic reticulum. The appearance of radioactivity in the rough endoplasmic reticulum and in the secretory granules indicates that a substantial amount of the precursor was incorporated into secretory protein. No doubt some of the ^{3}H-threonine was also incorporated into nonsecretory

protein, which presumably accounts for the labeling of other organelles such as mitochondria and nuclei.

In only one previous study (Jamieson and Palade, 1971) was an alteration in the secretory route with stimulation found. Unlike most other tissue types, in the unstimulated guinea pig pancreas the secretory protein appeared to bypass the Golgi cisternae (Jamieson and Palade, 1967). In the stimulated state, the more common route through the Golgi cisternae stacks was followed (Jamieson and Palade, 1971). Perhaps the unstimulated guinea pig pancreas is atypical in this respect.

Kinetics of the Secretory Process

The results of the present study show no change after stimulation in the time of *arrival* of the radioactivity in the organelles involved with protein secretion (Fig. 6). This is in agreement with the studies of Singh et al. (1973) and Jamieson and Palade (1971) in other systems. On the other hand, the increased rate of transport reported by Bieger et al. (1976) in the rat pancreas may be due to their use of a much longer period of stimulation (see review by Case, 1978). Slot et al. (1979) also observed accelerated transport of proteins in the pancreas of fed frogs in comparison with fasted animals. Perhaps the poikilothermic metabolism of the frog influences the response of protein synthesis and transport to stimulation in some way.

Rohr et al. (1967) performed an electron microscope autoradiographic study of the murine Brunner's gland in which the kinetics of the secretory process differed from those reported in the present study. The peaks of radioactivity in the rough endoplasmic reticulum, Golgi apparatus, and secretory granules occurred at 30, 15, and 60 minutes, respectively, compared to 5, 60, and 120 minutes in the present study. Several technical factors could contribute to this discrepancy. First, the dietary state of the mice was not described and might have been different in the earlier study. Second, Rohr et al. used direct grain counts, while the probability circle method was employed in the present study. Third, the selection of micrographs for analysis was standardized in the present study to ensure equal representation of basal and apical parts of the cell, which have very different contents of organelles (Geuze and Kramer, 1974; Helander, 1978; Ermak and Rothman, 1981), while this was apparently not the case previously. Finally, the number

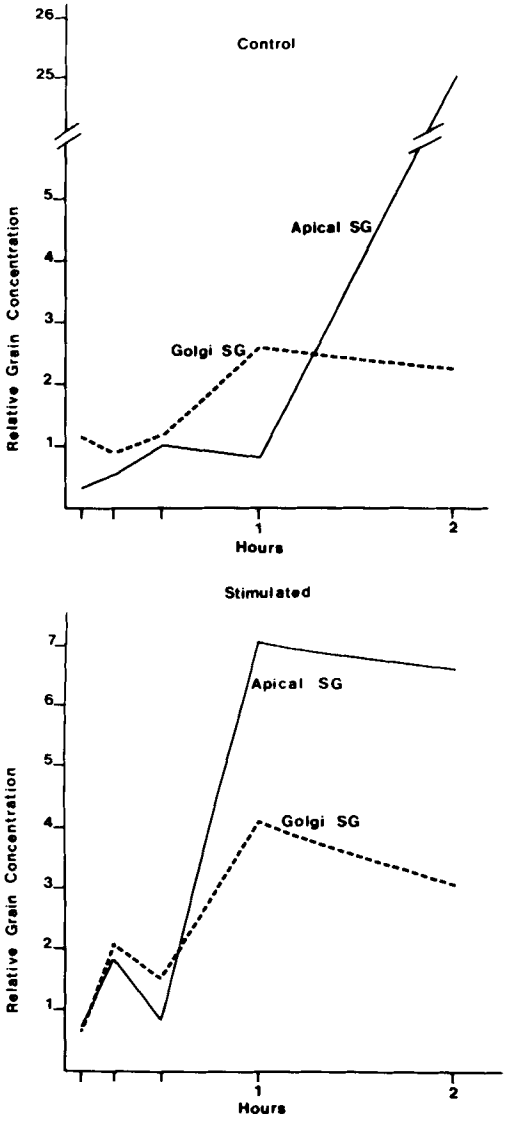

Fig. 8. The relative grain concentrations associated with different populations of secretory granules in control and stimulated animals. Golgi SG, Golgi-associated secretory granules; apical SG, apical secretory granules.

of animals and the extent of variation at each interval were not described by Rohr et al. (1967).

Labeling of Secretory Granules

Even though radioactivity began to increase noticeably in secretory granules between 30 minutes and 1 hour after injection of the precursor in both control and stimulated cells, the total percentage of silver

grains over secretory granules was less in stimulated than in control glands. There are at least two explanations for this observation. First, it probably reflects the smaller number of granules in the stimulated cells. In accord with this is the observation that the relative *concentration* of radioactivity in secretory granules (% grains/% volume) was actually higher in stimulated than in control cells at the 1-hour interval (Fig. 8). Second, drainage of radioactivity from the rough endoplasmic reticulum was slower in stimulated cells, as discussed further in the following section.

The sequential labeling of Golgi-associated and apical secretory granules in control cells likely reflects movement of granules from their site of formation in the Golgi apparatus to the cell apex. The paucity of apical secretory granules in the previously stimulated cells may facilitate the movement of newly formed granules from the Golgi region, thus accounting for the earlier peak in relative concentration of radioactivity in apical granules of stimulated cells (Fig. 8).

Drainage of Radioactive Protein From the Rough Endoplasmic Reticulum

The drainage of radioactive protein from the endoplasmic reticulum was slower in previously stimulated Brunner's glands than in nonstimulated glands. Even though the percentage of silver grains was within 0.5% in the two cases in the initial samples, by 2 hours after the injection of the radioactive precursor, the percentage of silver grains in the control cells was significantly lower than that in the stimulated cells. The slower drainage of radioactivity from the rough endoplasmic reticulum was also reflected in a less sharply defined peak of labeling of the Golgi apparatus. This finding was unexpected because previous authors reported either no change (Jamieson and Palade, 1971; Singh et al., 1973) or an increase (Bieger et al., 1976; Slot et al., 1979) in the rate of drainage of pancreatic rough endoplasmic reticulum with stimulation. (It should be noted, however, that the data of Jamieson and Palade show the percentage of autoradiographic grains over the rough endoplasmic reticulum for control and stimulated tissues within 1% of each other 3 minutes after the radioactive pulse, but a nearly 10% difference by 57 minutes, with the control being the lower value.)

Although the basis for the slower drainage of the rough endoplasmic reticulum in stimulated Brunner's glands is uncertain, some possible explanations for this unusual observation can be mentioned. First, exocytosis and the subsequent retrieval of excess plasma membrane might deplete a cellular component necessary for transport of secretory product from the rough endoplasmic reticulum. A second possibility is that there are two functional compartments within the rough endoplasmic reticulum. According to this hypothesis, the function of one compartment is storage, while the second compartment maintains a basal level of transport of secretory product. Discharge of secretory granules by stimulation would increase the demand for secretory product to replenish the granule population. If this promoted emptying of the storage compartment in the rough endoplasmic reticulum, then following recovery from stimulation the storage compartment would have to be restocked, resulting in retention of a greater proportion of newly synthesized protein within the rough endoplasmic reticulum.

Another possible explanation for the retention of radioactivity in the endoplasmic reticulum of stimulated cells is an increase in synthesis of proteins that are retained within the cell. The question of whether a change in rate of synthesis of intracellular and/or secretory proteins contributed to the alteration in labeling cannot be answered from the present study, however, because we compared the relative numbers of grains between parts of stimulated and unstimulated cells and did not compare absolute amounts of radioactivity. A comparison of absolute levels of radioactivity in radioautographs is difficult and requires that several technically demanding conditions be met, including uniformity of section thickness, dose of precursor, emulsion thickness, exposure time, etc. (Salpeter and McHenry, 1973). In any event, it seems unlikely that the greater percentage of grains over the rough endoplasmic reticulum of stimulated cells at later intervals resulted from continuing incorporation of label, since in a comparable experiment in which young rats were injected intravenously with ^{3}H-tyrosine, the acid-soluble radioactivity in plasma dropped precipitously between 2 and 10 minutes after administration, and fell to less than 10% of the initial value by 45 minutes (Nakagami et al., 1971).

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